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Current Asthma in Schoolchildren Is Related to Fungal Spores in Classrooms

Chi-Hsien Chen, MD; H. Jasmine Chao, PhD; Chang-Chuan Chan, PhD; Bing-Yu Chen, PhD; and Yue Leon Guo, MD, PhD

BACKGROUND: The presence of visible mold in households is associated with asthma. However, the role of "classroom fungus" in the development of childhood asthma, as well as the fungal species that may lead to asthma, remains controversial. This nationwide school survey was conducted to investigate the correlation between fungal spores in classrooms and asthma in schoolchildren.

METHODS: From April to May 2011, a cross-sectional survey was conducted to assess allergic/asthmatic conditions in schoolchildren aged 6 to 15 years old in 44 schools across Taiwan. Personal histories and current asthmatic conditions were collected using a modified International Study of Asthma and Allergies in Childhood questionnaire. Fungal spores in classroom were collected using a Burkard Personal Air Sampler and counted under light microscopy. Three-level hierarchical modeling was used to determine the complex correlation between fungal spores in classrooms and childhood asthma.

RESULTS: The survey was completed by 6,346 out of 7,154 parents (88.7%). The prevalences of physician-diagnosed asthma, current asthma, and asthma with symptoms reduced on holidays or weekends (ASROH) were 11.7%, 7.5%, and 3.1%, respectively. The geometric mean spore concentrations of total fungi, *Aspergillus/Penicillium*, and basidiospores were 2,181, 49, and 318 spores/m^3. *Aspergillus/Penicillium* and basidiospores were significantly correlated with current asthma and ASROH after adjusting for personal and school factors. Of those with current asthma, 41% reported relief of symptoms during weekends.

CONCLUSIONS: Classroom *Aspergillus/Penicillium* and basidiospores are significantly associated with childhood asthma and ASROH. Government health policy should explore environmental interventions for the elimination of fungal spores in classrooms to reduce the prevalence of childhood asthma.

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ABBREVIATIONS: ASROH = asthma with symptoms reduced on holidays or weekends

AFFILIATIONS: From the Department of Environmental and Occupational Medicine (Drs C.-H. Chen, Chan, and Guo), National Taiwan University College of Medicine and National Taiwan University Hospital; the School of Public Health (Dr Chao), Taipei Medical University; and the Institute of Occupational Medicine and Industrial Hygiene (Drs Chan, B.-Y. Chen, and Guo), National Taiwan University, Taipei, Taiwan.

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CORRESPONDENCE TO: Yue Leon Guo, MD, PhD, Department of Environmental and Occupational Medicine, National Taiwan University (NTU), College of Medicine and NTU Hospital, Room 339, 17 Syujhou Rd, Taipei 100, Taiwan; e-mail: leonguo@ntu.edu.tw

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Asthma is the most common chronic disease in childhood. It not only influences growth and disrupts schooling but also can lead to mortality and long-term health-care costs.[1] The impact of childhood asthma is tremendous and multifaceted. Thus, efforts to determine and eliminate modifiable causes are important.

Various environmental stimuli, including dust mites, animal dander, viruses, tobacco smoke, and air pollutants, have been reported as related to asthma.[1] However, the relationship between fungi and asthma has been inconclusive. Evidence that points to fungi as causal agents includes the higher number of airway symptoms (eg, coughing, phlegm, wheezing, sore throat, and runny nose) in children who live in damp and moldy dwellings[2] and the association of visible house molds with current asthma.[3-7] Moreover, environmental remedies for molds have been proven effective in reducing asthmatic symptoms.[8,9] Because of the ubiquitous distribution of molds, it is important to identify and characterize their health effects before any remediation can be applied.

However, information regarding the fungal species responsible for the development and attack of asthma remains inconsistent.[10,11] Simoni et al[12] have reported that children exposed to higher levels of molds are at increased risk of persistent cough. In addition, *Aspergillus* and *Penicillium* DNA have been positively correlated with wheezing.[12] Although another study investigating caregivers also disclosed similar results regarding *Aspergillus*,[13] other investigations have failed to demonstrate significant correlation between fungi and asthmatic symptoms.[14-19] This inconsistency may be partly due to biases in estimating the extent of environmental molds. For example, a simple questionnaire based on subjective observation alone may provide insufficient information,[13] whereas a culture-based assessment may miss fungi that do not grow on general culture media (ie, basidiospores).[10,20] Moreover, limited patient numbers in these surveys may also contribute to the incongruent results.

In the United States, dampness and visible molds in campuses have become an important public health issue for children.[21] However, fungi-related studies in schools are scarce. The Massachusetts Department of Public Health has reported a significant association between asthma prevalence and school mold conditions.[22] Other studies have also found positive correlations between excessive moisture/mold growth and the prevalence of asthmatic symptoms among schoolchildren.[23-25] These findings are mostly from self-report questionnaire surveys, and such environmental fungal assessments may lead to biased estimation. Furthermore, it is impossible to identify the actual causal species with such a study design.

To elucidate the actual correlation between fungal spores in classrooms and childhood asthma, this nationwide school survey was conducted in Taiwan. Elementary and junior high school students (N = 6,346) were enrolled. Numbers of fungal spores in 264 classrooms were collected from 44 schools across the country. Environmental fungal assessment was done by on-site air sampling and direct microscopic inspection to ensure better objective measurements of mold exposure.

Materials and Methods

Design and Study Population

Between April and May 2011, a nationwide cross-sectional survey was conducted in 44 schools in Taiwan. A modified Chinese version of the International Study of Asthma and Allergies in Childhood questionnaire was used for asthma assessment. One middle school and one elementary school were selected randomly from each administrative district for further study (Fig 1). Youngsters from the randomly selected one class per grade (elementary school level) and three classes from grades seven and eight (junior high school level) were the target population. The institutional review board of National Taiwan University Medical Center approved the study (reference number 201002051R), and the participants' parents provided informed consent.

Health Outcomes

Physician-diagnosed asthma was defined by caregivers' reports ("Has your index child ever been diagnosed asthmatic by a physician in her/his lifetime?") and from four questions investigating current asthma symptoms ("Has your index child ever had wheeze or whistling in the chest in the last 12 months?", "In the last 12 months, has your index child's chest sounded wheezy during or after exercise?", "In the last 12 months, has your index child had a dry cough at night, apart from a cough associated with a cold or chest infection?", and "In the last 12 months, has wheezing ever been severe enough to limit your index child's speech to only one or two words at a time between breaths?").

Current asthma was defined as "yes" answers for questions on physician-diagnosed asthma and current asthmatic symptoms. "Holiday improving" was assessed by the question "Have your index child's current asthmatic symptoms improved during holiday or weekend?" Asthma with symptoms reduced on holidays or weekends (ASROH) was defined as a "yes" answer to "holiday improving" among children with "current asthma."

Exposure Assessment

Fungal-spore sampling was conducted for 40 min continuously during class in the morning using the Burkard Personal Air Sampler (Burkard Manufacturing Co Ltd). The sampler was placed in the center of each classroom and at the height of children's respiratory zone. Fungal spores were identified via glycerin-jelly stain and microscopy. The concentration of fungal spores was calculated using a microscopically counted

number divided by the corresponding sampling volume in air and reported as spore number per cubic meter. Condition of visible mold on walls in classrooms was recorded while sampling.

Statistical Analysis

Logistic regression was used to examine the association between individual variables and health outcomes. Statistical analysis was done using the JMP software, version 5.0 (SAS Institute Inc).

To investigate the relationship between fungal spores and asthma/asthmatic symptoms, a three-level hierarchical logistic regression model was used. Statistical analysis was run by the commercial statistic software HLM 7 (Scientific Software International, Inc). The model evaluated variations among children (level 1), among classrooms (level 2), and among schools (level 3). Results were expressed as OR of interested outcomes for each fungal spore, which was obtained through the exponentiation of the slope coefficient.

Results

In the 44 selected schools, 7,154 children were assessed, and 6,346 children (88.7%; 3,028 boys and 3,318 girls) and their parents completed the survey. The response rates among schools varied from 86% to 93%. There were 808 who were excluded from the final analysis because they refused to participate in the study, had incomplete signature on informed consents, or had missing values on key questions.

Based on the demographic characteristics of the 6,346 participants (Table 1), the prevalences of physician-diagnosed asthma, current asthma, and ASROH were 11.7%, 7.47%, and 3.09%, respectively. In terms of the individual prevalence of asthma in the 44 schools (Fig 2A), younger subjects, male sex, parental atopy, and visible mold at home were associated with lifetime prevalence of physician-diagnosed asthma (Table 1). Maternal smoking during pregnancy was associated with increased risk of current asthma.

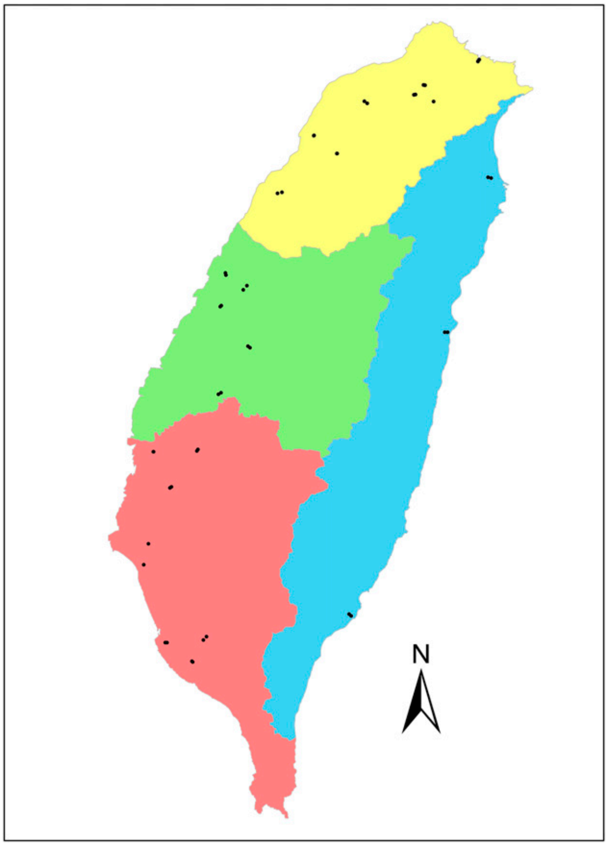

Figure 1 – *The 44 schools (dots) that participated in this study in Taiwan, 2011. Yellow, northern Taiwan; green, central Taiwan; red, southern Taiwan; blue, eastern Taiwan.*

Twenty-three morphologically identifiable fungal spores were counted and recorded in this investigation (Table 2). The spore concentration was distributed with great regional variety (Fig 2B). *Cladosporium* accounted for the major proportion of total fungal spores, followed by ascospores and basidiospore (Fig 2C). Only five fungal taxa were detectable in more than one-half of total classrooms. In the 264 classrooms, 18 (6.8%) have visible mold on walls. The spore concentration of *Aspergillus/Penicillium* and basidiospores was higher in classrooms with visible mold on walls (e-Table 1). None of the classrooms had animals, because they were not allowed in elementary and middle schools.

In the hierarchical logistic regression using the single fungal spore model, current asthma was associated with concentrations of ascospores, *Aspergillus/Penicillium*, and basidiospores in each classroom (Table 3), whereas ASROH was significantly related to concentrations of ascospores, *Aspergillus/Penicillium*, and basidiospores, and total fungal spores. Lifetime prevalence of physician-diagnosed asthma was not associated with any fungal spores (data not shown). In two fungal spore models, ascospores, *Aspergillus/Penicillium*, and basidiospores were included in the model for mutual adjustments, but only basidiospores and *Aspergillus/Penicillium* remained consistently associated with current asthma and ASROH.

Discussion

The present study has several important findings. First, similar to global trends,[26] the prevalence of childhood asthma in Taiwan has increased dramatically (6% in a previous survey and 11% in current study) in the past 10 years.[27] Second, *Aspergillus/Penicillium* and basidiospores are significantly associated with childhood current asthma and ASROH. To our knowledge, we are the first to demonstrate classroom basidiospores as an asthma-inducing factor. These associations are observed even

TABLE 1] Prevalence of Physician-Diagnosed Asthma, Current Physician-Diagnosed Asthma, and ASROH and the Associated Potential Risk Factors

		Ever Physician-Diagnosed Asthma		Current Physician-Diagnosed Asthma		ASROH	
Demographic	n	Diseased, %	OR (95% CI)	Diseased, %	OR (95% CI)	Diseased, %	OR (95% CI)
Total	6,346	11.7	...	7.47	...	3.09	...
Age, y							
< 10	1,551	13.63	1.33 (1.09-1.6)	9.27	1.22 (1.22-1.93)	3.24	1.15 (0.80-1.64)
10-14	1,844	11.81	1.13 (0.94-1.36)	7.98	1.04 (1.04-1.64)	3.38	1.20 (0.85-1.70)
≥ 14	2,951	10.64	1	6.22	1	2.84	1
Sex							
Male	3,028	14.07	1.61 (1.37-1.89)	8.91	1.60 (1.31-1.95)	3.72	1.60 (1.18-2.17)
Female	3,318	9.26	1	5.78	1	2.36	1
Parental education, y[a]							
< 13	2,916	10.83	0.87 (0.74-1.01)	6.38	0.86 (0.71-1.05)	3.01	0.96 (0.72-1.28)
≥ 13	3,288	12.30	1	7.84	1	3.13	1
Parental atopy							
Yes	1,557	17.85	2.93 (1.73-2.39)	11.47	1.98 (1.62-2.41)	5.48	2.46 (1.83-3.28)
No	4,789	9.67	1	6.14	1	2.30	1
Environmental tobacco smoke[a]							
Yes	2,605	11.34	0.95 (0.81-1.12)	7.62	1.07 (0.88-1.3)	3.48	1.3 (0.97-1.75)
No	3,406	11.86	1	7.16	1	2.69	1
Maternal smoking during pregnancy[a]							
Yes	159	16.13	1.50 (0.95-2.27)	11.61	1.70 (1.00-2.74)	6.45	2.26 (1.10-4.15)
No	5,924	11.40	1	7.16	1	2.96	1
Dog at home							
Yes	1,262	10.30	0.84 (0.68-1.02)	6.87	0.89 (0.69-1.13)	2.96	0.94 (0.65-1.34)
No	5,084	12.05	1	7.62	1	3.13	1
Cat at home							
Yes	195	9.33	0.77 (0.46-1.22)	6.22	0.82 (0.43-1.41)	3.63	1.19 (0.49-2.37)
No	6,151	11.78	1	7.51	1	3.08	1

(Continued)

TABLE 1] *(continued)*

Demographic	n	Ever Physician-Diagnosed Asthma		Current Physician-Diagnosed Asthma		ASROH	
		Diseased, %	OR (95% CI)	Diseased, %	OR (95% CI)	Diseased, %	OR (95% CI)
Cockroaches noted monthly at home[a]							
Yes	2,027	12.09	1.15 (0.97-1.37)	7.63	1.11 (0.90-1.37)	3.38	1.38 (0.99-1.94)
No	4,118	10.67	1	6.93	1	2.48	1
Air conditioner at home[a]							
Yes	5,816	11.63	1.02 (0.73-1.46)	7.34	0.93 (0.63-1.44)	2.97	0.65 (0.39-1.56)
No	336	11.45	1	7.83	1	4.52	1
Carpet at home[a]							
Yes	668	9.52	0.79 (0.59-1.05)	6.04	0.79 (0.56-1.09)	3.17	1.04 (0.54-1.61)
No	5,520	11.80	1	7.50	1	3.06	1
Water damage at home[a]							
Yes	618	12.48	1.11 (0.86-1.42)	8.37	1.19 (0.87-1.60)	2.96	0.99 (0.58-1.57)
No	5,530	11.38	1	7.14	1	3.00	1
Visible mold at home[a]							
Yes	1,661	13.66	1.30 (1.1-1.54)	9.45	1.50 (1.22-1.83)	4.39	1.72 (1.27-1.32)
No	4,487	10.82	1	6.53	1	2.60	1

ASROH = asthma with symptoms reduced on holidays or weekends.
[a]Numbers of subjects do not add up to total because of missing data.

when *Aspergillus/Penicillium* spores are at much lower concentrations compared with previous reports. Last, the stronger correlation between these fungal spores and "holiday improving asthma" (but not persistent asthma) may support the hypothesis that fungal spores in classrooms may be important risk factors for childhood asthma.

Methods for evaluating environmental mold have evolved in the past decades. Although fungal culture and molecular biology-based methods have been widely used in environmental surveys before, more recent reviews favor air sampling and direct spore counting (spores/m^3) for fungal assessments in health effect studies.[10] Limitations with traditional methods have led to such recommendations. For example, fungal culture has its strength in the ability to provide spore quantification and identify fungal species.[10] Nevertheless, this method may miss environmental nonviable spores, which are also allergenic,[28] and certain molds like basidiomycetes, which hardly grow with routine culture medium.[20] Another popular method is the measurement of fungal biomarkers, such as ergosterol or glucan. This method provides an alternative for fungal biomass assessments but fails to provide information regarding individual molds.[29,30] Nucleotide assays assess specific fungal DNA and detect both viable and nonviable molds. However, it is difficult to simultaneously cover a wide range of fungal species using this method.[12,14]

The current study applies the Burkard fungal spore trap with light microscopy examination to count spore concentrations of morphologically identifiable molds. This method is advantageous for its ability to assess both viable and nonviable fungal spores with a diverse spectrum of fungal species. However, its major limitation lies in its inability to differentiate between fungal species whose spores are morphologically alike, such as spores of *Aspergillus* species and *Penicillium* species.[31] Comparing all available methods today, air fungal sampling with microscopic examination remains the most suitable and feasible fungal assessment. Hence, this is used in the current survey.

Aspergillus and *Penicillium* can grow on a variety of indoor organic substrates. As such, they are important indoor fungi, especially in damp dwellings.[32-34] Human allergenicity to *Aspergillus/Penicillium* spores has been documented in previous research.[33] Today, there are at least 30 antigens from *Aspergillus* species and 17 antigens from *Penicillium* species listed as allergens by the International Union of Immunologic Societies.[35] Previous research has discovered that *Aspergillus/Penicillium* spores may trigger both immediate and delayed bronchial responses.[36,37] Moreover, *Aspergillus* and *Penicillium* are associated with asthmatic symptoms,[13,38] excess asthmatic symptom days,[39] and higher peak expiratory flow variability.[40,41] However, whether *Aspergillus/Penicillium* results in both asthma development and attacks or merely induces symptomatic exacerbation remains unknown.

Another unresolved issue regarding *Aspergillus/Penicillium* is that we are not sure whether the low level of spores in most indoor settings increases asthmatic symptoms.[13,18,39,42] Li et al[13] has found significant correlations between indoor fungal spore and sick-building syndrome (ie, nasal congestion, cough, phlegm, lethargy, and fatigue) among day-care center workers. In their study, the geometric means for *Aspergillus* and *Penicillium* spores were 31.5 and 275 colony-forming unit/m^3, respectively. In the present study, a similar correlation is already noted even when *Aspergillus* and *Penicillium* together have a spore level as low as 49.3 spores/m^3. Along with the observation that children have higher prevalence of fungus-related asthma,[10] it may be hypothesized that children are more vulnerable to molds and that they are prone to fungus-related asthma. However, further studies are warranted to confirm this hypothesis.

Another noteworthy mold in this study is the basidiomycete. Basidiomycete is a subdivision of fungus characterized by their specialized meiotic process and basidium formation. There are now approximately 20,000 to 25,000 species of basidiomycetes identified worldwide, most of which come from mushrooms, puffballs, or bracket fungi,[20] and, thus, they are abundant in outdoor air.[43] Although outdoor trees may be the origin for classroom basidiospores, indoor sources could not be totally excluded. Studies regarding the indoor origin of basidiospores are scarce, but wooden furniture with fungal destruction may be a substantial source.[43] Most Taiwan schools use wooden desks, chairs, and cabinets in the classrooms. This may help explain the source for basidiospores in the present study. Further studies to confirm the source for classroom basidiospores are warranted.

Allergenicity of basidiospores has been reported.[20] For those allergic to basidiomycetes, spore exposure may provoke both immediate and delayed airway responses.[44] Studies from New Orleans[45] and New Zealand[46] reveal a positive correlation between outdoor basidiospore

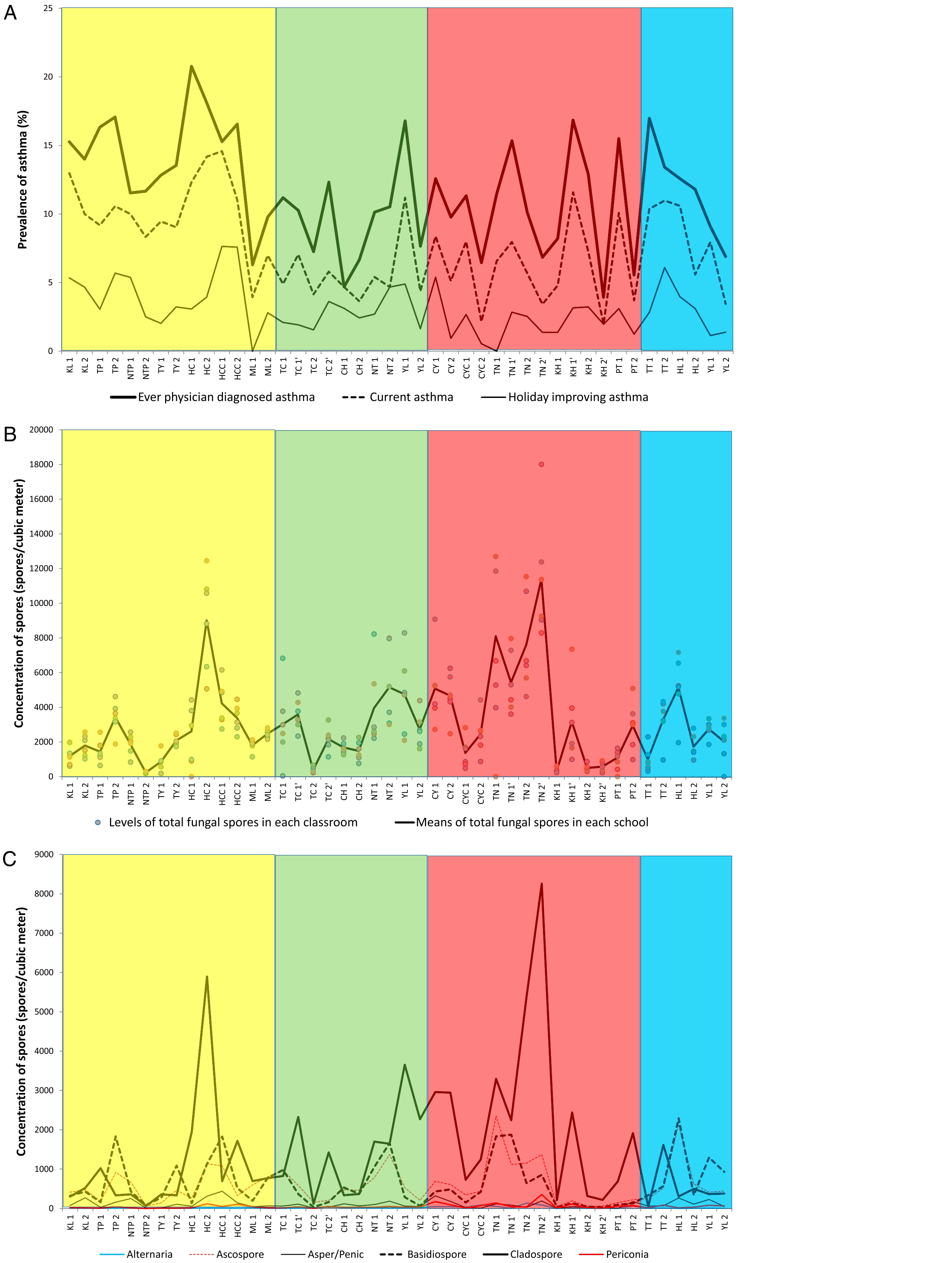

Figure 2 – *Distribution of asthma status and fungal spore concentrations in the 44 schools in 2011. A, Prevalence of ever having physician-diagnosed asthma, current asthma, and asthma with symptoms reduced on holidays. B, Levels of total fungal spores in classroom and their school means. C, Mean school levels of common fungal spores (detectable in > 50% of classrooms). The number after the city name denotes the school level: "1" for elementary school, and "2" for high school. Abbreviation of city names: Northern Taiwan: HC = Hsinchu City; HCC = Hsinchu County; KL = Keelung City;*

TABLE 2] Descriptive Statistics for Concentrations of Fungal Spores in 264 Classrooms

Exposure, Fungal Spores/m³	% Detect	Median[a]	IQR[a]	Geometric Mean[a]	Geometric SD[a]
Alternaria	58.71	8	15.2	10.45	3.04
Arthrium	41.67	4	9.7	7.29	2.4
Total ascospores	100.00	449	362.4	366.15	3.21
Aspergillus and *Penicillium*	87.12	52	97.1	49.3	3.97
Total basidiospores	99.62	394	455.2	318.01	3.84
Botrytis	7.58	4	0.4	4.41	1.59
Cercospora	16.67	4	0.5	4.89	1.75
Cladosporium	100.00	751	1,186.8	677.9	4.04
Curvularia	50.38	6	10.3	8.96	3.03
Drechslera and *Helminthosporium*	34.09	4	3.5	5.98	1.99
Epicoccum	1.52	4	0.4	4.01	1.21
Fusarium	2.65	4	0.4	4.03	1.19
Nigrospora	44.32	5	11.2	8.73	3.03
Oidium/Erysiphe	0.76	4	0.4	3.96	1.13
Periconia	81.06	25	31.4	22.59	3.61
Peronospora	18.94	4	0.6	5.14	1.86
Pithomyces	12.50	4	0.4	4.43	1.43
Polythrincium	1.89	4	0.4	3.99	1.15
Rusts	14.02	4	0.5	4.59	1.63
Smuts	35.61	4	12	8.67	3.52
Stemphylium	13.64	4	0.4	4.48	1.46
Tetraploa	3.41	4	0.4	4.07	1.27
Torula	37.12	4	4.2	6.5	2.23
Ulocladium	2.65	4	0.4	4.03	1.17
Other	78.79	38	41.1	28.34	3.89
Unidentified	75.76	16	25.9	16.63	3.16
Total fungal spore count	100.00	2,389	1,681.7	2,181.34	2.54

The detection rate of specific fungal spores was calculated by dividing the number of classrooms with microscopically identifiable spores by the number of all sampling sites (here, 264). IQR = interquartile range.
[a]Concentration of microscopically undetected fungal spores was estimated using 0.5 spore (one-half of detection limit) divided by its corresponding sampling volume of air.

level and asthma admissions. Although studies showing direct relationship between indoor basidiospores and asthma have been relatively lacking, effects of these spores on asthma are considered biologically plausible.

To our knowledge, the present study is one of the first to demonstrate a correlation between classroom basidiomycetes and childhood asthma. The mean spore concentration of basidiospores in this study is 632 spores/m³ (geometric mean, 318 spores/m³; minimum, 0; maximum, 3,234). In contrast, school surveys in the United States using a similar sampling method reveal much lower basidiospore concentrations (38 spores/m³ in Santa Fe and 246 spores/m³ in Kansas City).[47] Since Taiwan is located in the tropical/subtropical area, the oceanic climate is likewise the cause for the high basidiospore concentration uncovered in this survey. The results from this present study indicate that basidiospores may be an important cause of asthma in areas with high humidity and temperature.

ML = Miaoli County; NTP = New Taipei City; TP = Taipei City; TY = Taoyuan County. Middle Taiwan: CH = Changhua County; CY = Chiayi City; NT = Nantou County; TC = Taichung City; YL = Yunlin County. Southern Taiwan: CYC = Chiayi County; KH = Kaohsiung City; PT = Pingtung County; TN = Tainan City. Eastern Taiwan: HL = Hualien County; TT = Taitung County; YL = Yilan County.

TABLE 3] Effects of Fungal Spores on Current Asthma and ASROH

Fungal Spores	Current Asthma		ASROH	
	aOR[a]	95% CI	aOR[a]	95% CI
Single-fungus model[b]				
Alternaria	0.89	0.04-0.20	0.98	0.76-1.26
Ascospores	1.30[c]	1.01-1.67	1.40[c]	1.02-1.91
Aspergillus and *Penicillium*	1.41[d]	1.17-1.69	1.52[d]	1.17-1.97
Arthrium	0.88	0.68-1.12	1.39	0.96-2.02
Basidiospores	1.45[d]	1.12-1.81	1.66[d]	1.23-2.23
Cladosporium	1.05	0.88-1.26	1.15	0.89-1.48
Curvularia	1.03	0.78-1.35	1.10	0.82-1.48
Drechslera and *Helminthosporium*	0.71	0.48-1.04	0.63	0.36-1.08
Nigrospora	1.05	0.89-1.23	1.14	0.87-1.50
Periconia	0.89	0.77-1.03	0.99	0.78-1.27
Smuts	0.99	0.85-1.15	1.05	0.76-1.45
Torula	0.78	0.57-1.08	1.02	0.65-1.58
Total fungal spore	1.25	0.93-1.67	1.52[c]	1.04-2.21
Two-fungus model[b]				
A				
Ascospores	1.04	0.79-1.38	1.19	0.80-1.78
Aspergillus and *Penicillium*	1.39[d]	1.13-1.71	1.42[c]	1.09-1.85
B				
Ascospores	0.84	0.56-1.27	0.82	0.50-1.35
Basidiospores	1.61[d]	1.18-2.20	1.88[d]	1.22-2.91
C				
Aspergillus and *Penicillium*	1.28[c]	1.02-1.60	1.28[c]	1.01-1.62
Basidiospores	1.27[c]	1.01-1.59	1.44[c]	1.06-1.94

aOR = adjusted OR. See Table 1 legend for expansion of other abbreviation.
[a]The aOR was calculated for the effect of a log10 increase of spore concentration on asthma outcomes.
[b]Models were adjusted for children's age, sex, parental atopy, maternal smoke during pregnancy, visible mold at home, and schools. The relationship between fungal exposure and asthma status was examined only for those fungal spores with detection rate ≥ 30%.
[c]$P < .05$.
[d]$P < .01$.

In addition to triggering allergic reactions, mold could induce nonallergic response or toxic effect on the respiratory system.[10] The aerodynamic diameter of *Aspergillus/Penicillium* spores ranged from 2 to 3 μm,[10] and those of many basidiospores are < 10 μm,[10] rendering these spores inhalable and with the potential of inducing nonallergic airway response. Macrophages engulf inhaled spores and release proinflammatory cytokines, such as IL-8, which further recruit neutrophils and increase nonallergic airway inflammation.[10] Furthermore, mycotoxins attached to airborne fungal spores or fragments might induce respiratory toxicity.[48] Aflatoxins and ochratoxin produced by *Aspergillus* and *Penicillium* could be detected in indoor environments.[49] Ochratoxin has been reported to induce respiratory irritation in humans and pulmonary edema in rabbits.[50] Further research is needed to clarify the nonallergic effect of fungal spores and the role of attached mycotoxins in patients with asthma.

Outdoor spores penetrate into buildings readily.[10] Fungal spores in this study should have been mainly contributed by outdoor sources, because all schools in this study had windows opened during the assessments, which had been a common practice in most, if not all, of the elementary and middle schools in Taiwan. In addition, schoolchildren in this study spend 80% of their time indoors. Even if the outdoor fungal counts were larger or smaller than those indoors, the misclassification effects should have been relatively small.

Only a few studies reported outdoor fungal concentration in Taiwan. Two studies used similar sampling and counting methods as ours. One of them was conducted during 1993 to 1996 in Hualien, a city of eastern Taiwan, and reported outdoor levels of total fungal spores, *Cladosporium*, and ascospores in May to be approximately 8,000, 700, and 600 spores/m^3, respectively.[50] The same study reported an annual mean *Aspergillus/Penicillium* level of 184 spores/m^3.[51] The other study was conducted between December 2000 and April 2001 in Tainan, a city of southern Taiwan, and reported mean outdoor levels of total fungal spores, *Cladosporium*, ascospores, basidiospores, and *Aspergillus/Penicillium* to be 28,684, 19,834, 2,481, 1,273, and 247 spores/m^3, respectively.[52] These findings were of the same order ranges as observed levels of fungal spores in classrooms in this study.

To strengthen the causal inference in this study, the concept of "symptomatic relief away from exposure," a concept frequently used to confer work-related asthma in occupational medicine,[53] has been adopted. The question "Have your index child's current asthmatic symptoms improved during holiday or weekend?" was added to the questionnaire. In the final analysis, 41% of those with current asthma report relief of symptoms during the weekends, suggesting the potential role of schools as the source of allergens.

This study has several strengths. First, to our knowledge, this is one of the few nationwide surveys that investigated the correlation between airborne fungal spores and childhood asthma. Second, related studies carried out in school settings are scarce. Thus, this study may serve as a pilot investigation. Last, the fungal assessment method used in this survey provides the possibility of assessing both culturable and nonculturable fungi. Hence, the results here may be more reliable than those that used the traditional culture method.

However, this study also has several limitations. First, although moldy conditions at home could be an important factor for asthma,[54] mold counts at home were not directly measured because of the large number of subjects surveyed in this study. However, we believe it to be unlikely to change our conclusion. Our main findings are positive relation between current asthma (especially ASROH) and fungal spore counts of *Aspergillus/Penicillium* and basidiospores. Among those with home spore levels similar to or higher than those in the schools, such effects would not have been observed, thus, reducing the observed effects toward null. In addition, a previous study has found that households with visible mold on walls had higher indoor spore counts.[55] Self-reported visible mold on walls at home was adjusted in the regression models in this study. Thus, effects caused by unmeasured spore levels at home were adjusted. Second, because fungal sensitization was not assessed by skin prick or serologic tests, we were unable to determine whether the observed effects were due to allergic or nonallergic mechanisms. Although previous reports showed low prevalence of fungal sensitization in Taiwanese schoolchildren,[56,57] inappropriate fungal matrix used in current commercial kits had been considered as a potential problem for the low detection rates.[33] The findings from this current study underline the importance of developing measurements of immunologic markers more specific for regional fungal allergens, which would allow for more sensitive detection of atopic reaction to fungal allergens. Third, ambient levels of pollen were not measured in this study; thus, potential confounding effects of pollen on the observed fungal effect on asthma could not be totally ruled out. However, according to a large survey on Taiwanese children with allergy, the prevalence of pollen sensitization was very low, ranging from 1% to 2%,[56] mainly due to grasses.[58] The peak seasons of grass pollens are summer and autumn,[59,60] different from those of fungal spores (winter and spring).[61] Therefore, although the confounding effects of pollens could not be completely ruled out, they should be minimal. Fourth, our fungal measurements cannot distinguish *Aspergillus* from *Penicillium*, since their spores look almost identical under light microscopy. Because the two fungal species share common indoor substrates, this will not affect the strategy for fungal remediation in classrooms. However, previous study showed *Aspergillus* as a common mold in outdoor air, and only 6.8% of classrooms had visible mold on walls in this current study. Methods to control outdoor spore penetration (ie, closing windows during times of high outdoor spore levels) may have to be considered as well. Further study is needed to differentiate the effects between *Aspergillus* and *Penicillium*. Fifth, because of feasibility and budget concerns, the asthma defined in this study is self-reported asthma, without further confirmation tests. To resolve such a problem, a validated questionnaire is used, as well as "physician-diagnosed asthma" with information adopted from parents. This minimizes the possibility of diagnosis misclassification.

In conclusion, classroom *Aspergillus/Penicillium* and basidiospores are significantly correlated with childhood asthma. The effect level for *Aspergillus/Penicillium* to be associated with asthma has been lower than previously reported. The actual causal relationship between the two warrants further confirmation. The current findings may provide information for future meticulous evaluation and development of preventive strategy for asthma.

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